

## Four new earthworms of the *Amyntas aeruginosus* species group (Oligochaeta: Megascolecidae) from Nam Xam NBCA, Laos

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### Four new earthworms of the *Amyntas aeruginosus* species group (Oligochaeta: Megascolecidae) from Nam Xam NBCA, Laos. -

Earthworm specimens collected from the Nam Xam National Biodiversity Conservation Area, Laos belong to four new species of megascolecid earthworms: *Amyntas banlaoensis* sp. nov., *Amyntas khamlai* sp. nov., *Amyntas phadeangensis* sp. nov., and *Amyntas naphopensis* sp. nov. All four new species key to the *aeruginosus* group in Sims & Easton (1972), defined by having spermathecal pores in 7/8/9 and simple intestinal caeca. *Amyntas banlaoensis* sp. nov. has male pores superficial near lateral margins of XVIII in the 12<sup>th</sup> setal lines, on short columnar 0.3-0.5 mm diameter porophore, and tubercular genital markings paired in VII or VIII. *Amyntas khamlai* sp. nov. has male pores on retractable, eversible round porophores near the lateral margins of the ventrum in XVIII, and circular genital markings in paired postsetal linear groups of two or three on trailing edges of VII and VIII. *Amyntas phadeangensis* sp. nov. has male pores on alate porophore swellings composed of concentric rings, with paired genital papillae medial to the male porophores and embedded in the concentric rings. It has paired postsetal genital markings in 8<sup>th</sup> - 9<sup>th</sup> setal lines of VIII and IX. *Amyntas naphopensis* sp. nov. has male pores in small invaginations partly covered by raised U-shaped flap concave medially, and lacks other genital markings.

**Keywords:** Earthworms - Megascolecidae - Oligochaeta - Laos - Nam Xam NBCA - taxonomy.

### INTRODUCTION

Like many countries in Southeast Asia, relatively little is known about the earthworms of Laos. A few parts of Laos have been studied with regard to the taxonomy of Megascolecidae (Thai & Samphon, 1988; 1989; 1990b; Hong *et al.*, 2008). Thai and Samphon also reported earthworm distribution data from several regions (Thai & Samphon, 1990a; 1991ab), for a total of 59 Megascolecidae in *Metaphire*, *Pithemera* and *Amyntas*. Thai & Samphon (1990a; 1991ab) placed all the Laotian worms of these genera in *Pheretima* Kinberg, 1867, but *Pheretima* had been revised by Sims & Easton (1972). Species with intestinal caeca originating in XXVII and lacking both copulatory pouches and nephridia on spermathecal ducts were placed in *Amyntas*.

Taking into account this revision, *Amynthas* is the most diverse genus in the natural forests of northern Laos. Our recent collections are adding support to this statement.

During August 2004, collections were made in Nam Xam National Biodiversity Conservation Area (NBCA) in northern Laos, about 900 kilometres from Vientiane. Collections were made in the Important Bird Area (IBA), a higher-quality habitat zone located inside Nam Xam NBCA. The topography of the IBA is dominated by hills and low mountains, with a few peaks over 1,600 m elevation. The natural vegetation of the IBA comprises dry evergreen forest and mixed deciduous forest, with *Fokienia* forest at higher elevations, particularly in the west of the IBA, where most of the area is covered by evergreen forest on limestone. There are also some small areas of stunted, mossy upper montane forest at the highest elevations. Within Nam Xam NBCA, there are also significant areas of agricultural land and secondary re-growth following shifting cultivation, although these are mainly excluded from the IBA. The specimens were found in litter layers and soils in forests by digging and hand sorting.

In this paper, four species new to science are reported from Northern Laos. In Laos, *Amynthas* with two pairs of spermathecal pores come in two groups, those with the pores in 5/6 and 6/7, and those with the pores in 7/8 and 8/9. All four new species recorded here have pores in 7/8-8/9, corresponding to the *aeruginosus* group in Sims & Easton (1972).

Holotypes and paratypes are deposited in the Biology Department, Faculty of Science, National University of Laos, Vientiane (BDNUL). Paratypes are deposited in the Museum of Natural History of Geneva (MHNG), and the Korean Bioresources Collection, National Institute of Biological Resources (NIBR).

## DESCRIPTIONS

FAMILY MEGASCOLECIDAE ROSA, 1891

Genus *Amynthas* Kinberg, 1867

*Amynthas banlaensis* sp. nov.

Figs 1A-B

HOLOTYPE: Clitellate (BDNUL 0009); Laos, Hoaphane province, Namxam NBCA, Xamtai district, South- Houa Heunh, BanLao village (20° 00.821' N, 104° 40.623' E), 448 m, mature forests 3 km from local village near cemetery, in soil and litter layers, 2 September 2004, K. Inkhavilay coll.

PARATYPES: 1 semiclitellate (BDNUL 0013), 1 clitellate (MHNG INVE 68954), 1 clitellate (NIBRIV0000184280), same data as for holotype.

OTHER MATERIAL: 3 semiclitellates, 5 a clitellates, same data as for holotype.

ETYMOLOGY: The species is named for its type locality near the village of Ban Lao.

DIAGNOSIS: Spermathecal pores two pairs in 7/8/9 at 11<sup>th</sup> setal lines; male pores superficial near lateral margins of ventrum in XVIII at 12<sup>th</sup> setal lines, on short columnar 0.3-0.5 mm round porophore, base of porophores in shallow parietal invagination.

DESCRIPTION: Dimensions 83-150 mm by 6.4-6.5 mm at segment X, 5.0-6.5 mm at segment XXX, 5.0-6.4 mm at clitellum; body cylindrical, segments 85-113. Setae regularly distributed around segmental equators, numbering 40-47 at VII, 48-52 at XX,

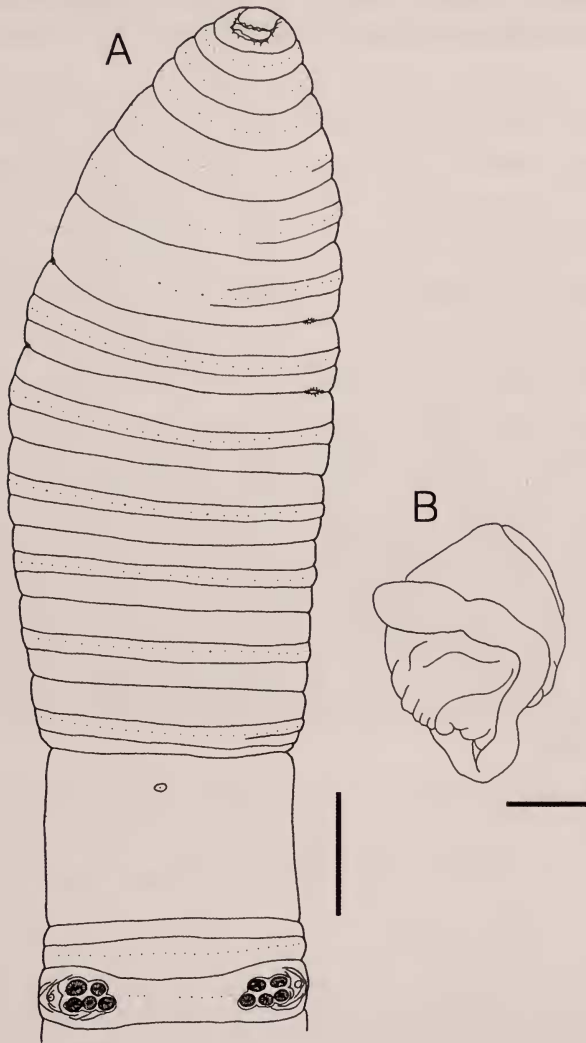


FIG. 1

*Amynthes banlaensis* sp. nov. (A) Ventral view. (B) Spermathecae and diverticulum. Scale bars = 2.5 mm (A), 1 mm (B).

5-12 between male pores, setal formula AA:AB:ZZ:YZ= 4.5:3:4:3 at XIII. Female pore single in XIV, on 0.2-0.3 mm oval. Prostomium epilobic with tongue open. Brown dorsal pigment, clitellum coffee color, formalin preservation. First dorsal pore at 12/13. Clitellum annular XIV-XVI; setae invisible externally.

Male pores superficial near lateral margins of ventrum in XVIII at 12<sup>th</sup> setal lines, on short columnar 0.3-0.5 mm round porophores, base of porophores in shallow parietal invagination. Between male pores two groups of papillae, each containing 2-4 circular presetal genital papillae, 1-3 postsetal genital papillae. Tubercular genital

markings paired VII or VIII, some in the middle of ventrum and some at lateral margins. Spermathecal pores two pairs in 7/8, 8/9 at 11<sup>th</sup> setal lines at lateral margins of ventrum.

Septa 5/6-7/8 thick, 8/9-9/10 absent, 10/11-13/14 thick. Gizzard in VIII-X. Intestine begins in XV, lymph glands from XXV. Typhlosole origin in XXV, full size XXVIII, ¼ lumen diameter. Intestinal caeca simple big finger-shaped sac with 5-6 short pockets on ventral margins, originating at XXVII, and extending anteriorly about to XXIV. Hearts in X-XIII. Male sexual system holandric, testes and funnels in ventrally joined sacs in X, XI. Seminal vesicles large pairs in X-XII, well developed, with ovate dorsal lobes in shallow clefts. Prostates well developed XVIII extending to XVII-XXII, each containing about 5-6 lobes partly attached to body wall. Crowded small stalked mushroom-shaped genital papillae glands in XVIII.

Ovaries in XIII. Paired spermathecae in VII, VIII; VIII bigger than VII, each ampulla is large, round but flattened by gizzard; short, stout muscular duct, shorter than ampulla, diverticulum chamber sausage-shaped, stalk short, no nephridia on spermathecae ducts. Short-stalked genital marking glands leaf-shaped in VII, VIII.

REMARKS: *Amyntas banlaoensis* sp. nov. keys to the *aeruginosus* group in Sims & Easton (1972). In this group, the following Laos species are recorded: *Amyntas choanus* (Thai & Samphon, 1988), *Amyntas otoformes* (Thai & Samphon, 1989), and *Amyntas uanus* (Thai & Samphon, 1990). *Amyntas otoformes* and *Amyntas uanus* have male fields very unlike the other *aeruginosus*-group species in Laos. *Amyntas choanus* has only one circular genital papilla in the male pore region, and is of smaller size (52 mm by 2 mm) than *Amyntas banlaoensis* sp. nov. (83-150 mm by 6.4-6.5 mm).

The new species is similar to *A. omeimontis* (Chen, 1946), with respect to the length, segments, setae, first dorsal pore, and type of caeca. It differs from *A. omeimontis* which has two groups of genital papillae, three to seven genital papillae surrounding the depression of male pore region, and another group of two to seven on antero-medial side of the depression in front of setal circle, but *Amyntas banlaoensis* sp. nov. has two to four circular genital papillae and one to three postsetal papillae between the male pores. In *A. omeimontis* there are about 40 to 50 small genital markings closely arranged in rows covering the setal zone of the ventral side of 11<sup>th</sup> segment and anteriorly to intersegmental furrow 10/11, but the new species has no genital marking outside the spermathecal pore region. The spermathecae of *Amyntas banlaoensis* sp. nov. have ducts shorter than the ampullae, but *A. omeimontis* has ducts longer than ampullae.

*Amyntas khamlai* sp. nov.

Figs 2A-B

HOLOTYPE: Clitellate (BDNUL 0010), Laos, Hoaphane province, Namxam NBCA, Xamtai district, Hoae Du mountain (20° 01.040' N, 104° 39.205' E), 384 m, thin layer soil with some leaf litter, secondary forest with bamboos and trees, 5 km from local village, in soil and litter layers, 12 September 2004. K. Inkhavilay coll.

PARATYPES: 1 semiclitellate (BDNUL 0014), 1 semiclitellate (MHNG INVE 68955), 1 semiclitellate (NIBRIV0000184281), same data as for holotype.

OTHER MATERIAL: 1 acitellate, same data as for holotype.

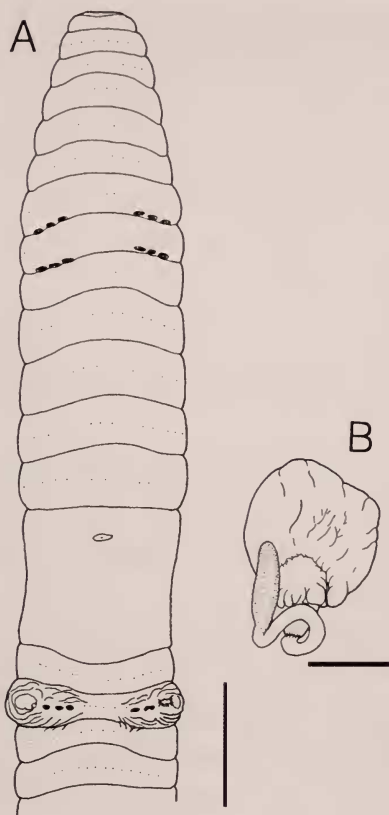


FIG. 2

*Amynthus khamlai* sp. nov. (A) Ventral view. (B) Spermathecae and diverticulum. Scale bars = 3 mm (A), 4 mm (B).

ETYMOLOGY: The species is named for the collector, Khamla Inkhavilay.

DIAGNOSIS: Spermathecal pores paired in 7/8/9 between 7<sup>th</sup> and 8<sup>th</sup> setal lines; male pores on retractable, eversible round porophores in XVIII at 11<sup>th</sup> setal lines.

DESCRIPTION: Dimensions 65-81 mm by 4.5-4.6 mm at segment X, 4.4-4.5 mm at segment XXX, 4.1-4.5 mm at clitellum; body depressed, segments 110-114. Setae regularly distributed around segmental equators, numbering 38-40 at VII, 48-50 at XX, 7-13 between male pores, setal formula AA:AB:ZZ:YZ= 3:2.5:3:2.5 at XIII. Female pore single in XIV, on 0.2-0.3 mm oval porophore. Prostomium epilobic with tongue open. Brown dorsal pigment, yellowish ventrally, clitellum coffee color, formalin preservation. First dorsal pores at 11/12. Clitellum annular XIV-XVI; setae invisible externally.

Male pores on retractable, eversible round porophores near lateral margins of ventrum in XVIII at 11<sup>th</sup> setal lines, surrounded by thickened half-circle connected to elevated zone bearing genital papillae, 4.0-4.5 mm between male pores. Between male pores paired linear groups of 2-4 postsetal round genital papillae; lateral-most papillae

on retractable porophore with male pore. Spermathecal pores paired in 7/8-8/9 between 7<sup>th</sup> and 8<sup>th</sup> setal lines, lateral to genital markings, on small bumps at trailing edges of VII, VIII. Genital markings paired linear groups of 2-3 postsetal circles in VII, VIII on posterior edges; sometimes in VI.

Septa 5/6-7/8 thick; 8/9-9/10 absent; 10/11-12/13 thin. Gizzard globular and large in VIII-X. Intestine begins in XVI, lymph glands found from XVIII. Typhlosolar fold < 1/3 lumen from XXVII. Intestinal caeca simple, originating in XXVIII, extending anteriorly about to XXIV, finger-shaped with serrate ventral margin. Hearts X-XIII. Male sexual system holandric, testes and funnels in ventrally joined sacs in X-XI. Seminal vesicles XI-XII with small dorsal lobe set deeply in a dorsal cleft. Prostates in XVIII between XVI-XXI divided into many short, deeply divided lobes, duct muscular, S-curved. Genital papillae glands with long slender stalks and small glandular heads in XVIII, numbers of glands corresponding to numbers of genital papillae.

Ovaries in XIII. Paired spermathecae preseptal in VIII, postseptal in IX, broad lenticular cordate ampullae, diverticulum slender stalk as long as ampulla with chili-shaped chamber, no nephridia on spermathecal ducts. Genital marking glands same structure as in XVIII, numbers of glands corresponding to numbers of genital markings in VII, VIII close to spermathecal ducts.

REMARKS: This species also keys to the *aeruginosus* group in Sims & Easton (1972), within which *Amyntas khamlai* sp. nov. is distinguished by the patterns of genital papillae, and genital markings. *Amyntas khamlai* sp. nov. is similar to *Amyntas phadeangensis* sp. nov., with respect to body length, spermathecal pores, and presence of genital markings, but the details are different. *Amyntas khamlai* sp. nov. has genital markings in paired linear groups of 2-3 postsetal in VII, VIII on trailing edges of those segments, but *Amyntas phadeangensis* sp. nov. has paired postsetal genital markings in VIII, IX closer to the segmental equators. *Amyntas khamlai* sp. nov. has far fewer setae between the male pores (7-13 vs. 39-42). The spermathecal pores in *Amyntas khamlai* sp. nov. are between the 7<sup>th</sup> and 8<sup>th</sup> setal lines, but those of *Amyntas phadeangensis* sp. nov. are in the 13<sup>th</sup> setal lines. *Amyntas khamlai* sp. nov. has the intestinal origin in XVI as opposed to XV for *Amyntas phadeangensis* sp. nov. This new species is similar to *A. robustus* (Perrier, 1872), and *A. arrobustus* (Thai, 1984), but it differs from them in the characters of the male pore region. The genital papilla of *A. arrobustus* is single and presetal in XVIII, but *Amyntas khamlai* sp. nov. has 2-4 postsetal papillae. The new species is similar to *A. robustus*, but is separated easily by the genital markings of spermathecal pores. Genital markings of *A. robustus* are small, only one intersetal interval wide and are present inside the spermathecal porophore, and in one or more of the following locations within VII and/or VIII: just in front of the spermathecal pores, just behind the spermathecal pores, or median to the pores and presetal (Gates, 1972).

***Amyntas phadeangensis* sp. nov.**

Figs 3A-B

HOLOTYPE: Clitellate (BDNUL 0011); Laos, Hoaphane province, Namxam NBCA, Xamtai district, Pha Deang mountain, Ban Lao village (20° 00.727' N, 104° 39.894' E), 681 m, secondary forest with small trees and some bamboos 10 km from village, in soil and litter layers, 3 September 2004, K. Inkhavilay coll.

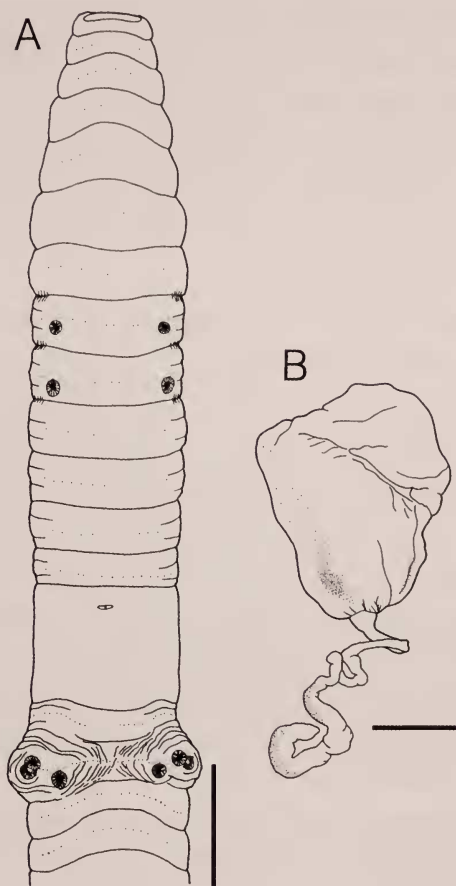


FIG. 3

*Amynthus phadeangensis* sp. nov. (A) Ventral view. (B) Spermathecae and diverticulum. Scale bars = 4 mm (A), 2 mm (B).

PARATYPE: 1 semiclitellate (BDNUL 0015), same data as for holotype.

OTHER MATERIAL: 1 acitellate, same data as for holotype.

ETYMOLOGY: The species is named for its type locality, Pha Deang mountain.

DIAGNOSIS: Spermathecal pores two pairs in 7/8/9 at lateral margins of ventrum 13<sup>th</sup> setal line; male porophores alate swellings composed of concentric rings; pores within small central porophore.

DESCRIPTION: Dimensions 50-110 mm by 4.0-5.0 mm at segment X, 4.4-4.7 mm at segment XXX, 4.0-6.0 mm at clitellum; body cylindrical throughout, segments 59-102. Setae evenly distributed around segmental equators, numbering 45-47 at VII, 60-65 at XX, 39-42 between male pore, setal formula AA:AB:ZZ:YZ= 3:3:4:3 at XIII. Female pore single in XIV, on 0.1-0.3 mm oval. Prostomium epilobic with tongue

open. Light brownish dorsal pigment, clitellum coffee color, formalin preservation. First dorsal pore at 12/13. Clitellum annular XIV-XVI; setae invisible externally.

Male pores near lateral margins of ventrum in XVIII, on alate swellings composed of concentric rings; pores within small central porophore. Paired genital papillae presetal, medial to male porophore; additional pair of genital papillae postsetal embedded in outer concentric rings, pore 0.2-0.3 mm in diameter. Spermathecal pores two pairs in 7/8, 8/9 at lateral margins of ventrum in 13<sup>th</sup> setal lines. Paired postsetal genital markings in 8<sup>th</sup> - 9<sup>th</sup> setal line VIII, IX; paired genital markings VIII nearly in furrow 8/9, median to spermathecal pores.

Septa 5/6-7/8 muscular, 8/9-9/10 absent, 10/11-12/13 slightly muscular. Gizzard in VIII-X. Intestine begins in XV, lymph glands not found. Typhlosole simple fold about 1/4 lumen diameter found from XXVII. Intestinal caeca simple, originating in XXVII, extending anteriorly about to XXII, finger-shaped with 8-10 pockets on ventral margin. Intestine deeply pouched 21-25, with small typhlosole ridge ending at 25/26, no other blood vessel ridges in this area. Esophageal hearts X-XIII. Male sexual system holandric, testes and funnels in ventrally joined sacs in X-XI. Seminal vesicle two large pairs in XI-XII, with small round dorsal lobes. Prostates in XVIII large between XVI-XXIII, each containing about 4-6 main lobes, thick muscular duct. Genital papillae glands sessile, very large in XVIII, two pairs; transverse muscle bands anterior to prostatic duct, covering anterior pair of genital papillae glands.

Ovaries in XIII. Paired spermathecae in VIII and IX, each ampulla large ovate to sagittate sacs, duct short and muscular without nephridia, some small black pigment dots on ampulla surface, diverticulum with muscular stalk, long kinked chamber, diverticulum shorter than ampulla. Three pairs large genital marking glands VIII-IX near spermathecae, very short stalked or sessile.

REMARKS: The species shares some internal characteristics of the prostates, seminal vesicles, septa, pigment, and esophageal hearts with *Amyntas khamlai* sp. nov. Distinctions were detailed above under *Amyntas khamlai* sp. nov. *Amyntas phadeangensis* sp. nov. has an unusual intestine, which is deeply pouched in XXI-XXV with a small typhlosolar ridge ending at 25/26. The present species appears to be closely related to *A. longisiphonus* (Qiu, 1988) from Guizhou, China with two spermathecal pores in 7/8 and 8/9, but it is separated easily by the shape of the male pore region and genital markings. *Amyntas longisiphonus* has 5-8 pairs of papillae on the ventral side in XVIII-XXII or XXV, but *Amyntas phadeangensis* sp. nov. has paired presetal papillae, and paired postsetal papillae in XVIII. The new species is different from *A. robustus* and *A. arrobustus* in respect to the shape of the male pore region and genital papillae, *Amyntas robustus* usually has two papillae just median to each male porophore. The male porophore is on a thick disk, and the spermathecal pores are near mid-lateral.

***Amyntas naphopensis* sp. nov.**

Figs 4A-B

HOLOTYPE: Clitellate (BDNUL 0012):Lao, Hoaphane province, Xamtai district, Namxam NBCA, Naphop mountain NBCA. (20° 01.588' N, 104° 39.422' E), 414 m, good forest with thick litter layer, 10 km from village near a stream, in soil and litter layers, 4 September 2004, K. Inkhavilay coll.



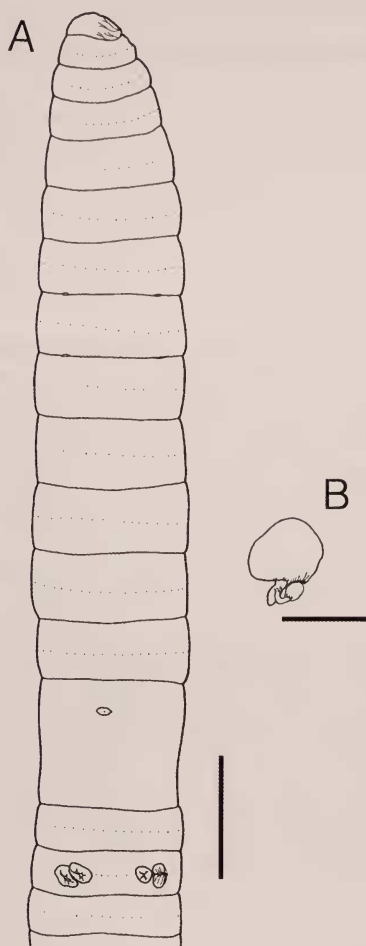


FIG. 4

*Amynthus naphopensis* sp. nov. (A) Ventral view. (B) Spermathecae and diverticulum. Scale bars = 3 mm (A), 2 mm (B).

PARATYPES: 1 semiclitellate (BDNUL 0016), 1 clitellate (MHNG INVE 68956), 1 clitellate (NIBRIV0000184282), same data as for holotype.

OTHER MATERIAL: 10 clitellates, same data as for holotype; 60 clitellates, Namxam NBCA, Pha tolk, (20° 00.552' N, 104° 40.369' E), 470 m, soil and litter layers, 6 September 2004, K. Inkhavilay coll.

ETYMOLOGY: The species is named for its type locality, Naphop mountain.

DIAGNOSIS: Spermathecal pores two pairs in 7/8/9 at 7<sup>th</sup> setal lines; male pores XVIII at 6<sup>th</sup> setal lines in small invaginations partly covered by raised U-shaped flap concave medially.

DESCRIPTION: Dimensions 58-67 mm by 3.5-3.7 mm at segment X, 3.4-3.7 mm at segment XXX, 3.5-3.6 mm at clitellum; body cylindrical, segments 49-66. Setae regularly distributed around segmental equators, numbering 38-39 at VII, 40-42 at XX, 4-10 between male pores, setal formula AA:AB:ZZ:YZ= 2:2:3:2 at XIII. Female pore conspicuous single in XIV, on 0.2-0.3 mm oval. Prostomium epilobic with tongue open. Dark brown dorsal pigment, lighter on dorsal setal rings, lighter brown ventrally, VII-IX unpigmented between spermathecal pores. Clitellum coffee color, formalin preservation. First dorsal pore at 5/6. Clitellum annular XIV-XVI; setae invisible externally. On post-clitellate segments, ventral-most section spanning DD in XX to FF by XL, elevated within segments, furrows thus deepened, ventrum ladder-like in appearance.

Male pores XVIII at 6<sup>th</sup> setal lines in small invaginations partly covered by raised U-shaped flap concave medially, enclosing two oval genital papillae, 2.2-2.5 mm between male pores. Spermathecal pores two large pairs in 7/8, 8/9 at 7<sup>th</sup> setal lines conspicuous near the lateral margins of ventrum, about 0.7-0.9 mm between spermathecal pores. Paired equatorial genital papillae, median to male pores on XVIII, circular or oval-shaped, but mostly circular; size variable. Genital markings lacking.

Septa 5/6/7/8 thick, 8/9-9/10 absent, 10/11-12/13 very thin. Gizzard in VIII-X. Intestine begins in XV, lymph glands not found. Typhlosole low fold from XXVII. Intestinal caeca simple, originating in XXVIII, extending anteriorly about to XXIV, finger-shaped with smooth margins or only segmental indentations. Hearts X-XIII. Male sexual system holandric, testes and funnels in ventral paired sacs in X-XI; sacs of a side united. Seminal vesicles two pairs large, without dorsal lobes in XI-XII, vesicle of XI extends into X. Prostates in XVIII between XVII-XIX each containing 3 main lobes, many small deeply divided secondary lobes, muscular ducts coiled in 540° spiral, spiraling proximal to distal counter-clockwise on right side and clockwise on left side; short non-muscular section from gland to spiral twisted opposite. Genital papillae glands and copulatory pouches not found.

Ovaries in XIII. Paired spermathecae in VII, VIII each ampulla large ovate to rounded angular, muscular duct very short, a little bit longer than diverticulum, short diverticulum egg-shaped chamber, no nephridia on the spermathecal ducts. Genital marking glands not found.

REMARKS: The present species keys to the *aeruginosus* group by the two spermathecal pores in 7/8-8/9. *Amyntas naphopensis* sp. nov. has spermathecal pores in 7/8 and 8/9, but spermathecae are in VII, VIII, so these are unusual preseptal spermathecae. This species has distinctive prostatic ducts coiled in 540° spirals, the spiraling directions opposite on the two sides of the body. Finally, the elevated ventral setal zones of post clitellar segments are also distinctive. Thus *Amyntas naphopensis* sp. nov. is easily distinguished from the other members of the species group.

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